IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 1687
Flack *et al.*	Art Unit: 1614
Appl. No.: 10/806,088	Examiner: Anderson, James D.
Filed: March 22, 2004	Atty. Docket: 225011
For: **Gossypol for the Treatment of Cancer**	

Declaration of Jon Theodore Holmlund, M.D. under 37 C.F.R. § 1.132

Commissioner for Patents
PO Box 1450
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Sir:

I, Jon Theodore Holmlund, M.D., declare and state:

1. I am Chief Medical Officer and Vice President at Ascenta Therapeutics, Inc., the licensee of the above-captioned application. A copy of my curriculum vitae and list of publications is attached hereto as Exhibit A.

2. I have read and understand the above-captioned application and its prosecution history, including the office action dated August 4, 2006. I understand that the examiner has rejected the pending claims of the application as not enabling the practice of the claimed methods for their full scope. In my opinion, it is expected and predictable that (-)-gossypol may be used to treat a wide diversity of cancers.

3. Recent findings support the expectation that (-)-gossypol is useful for the treatment of a wide diversity of cancers in humans. It has been reported that modulating apoptosis (programmed cell death) suppressing members of the Bcl-2 family holds potential for treating cancer. See Shore, G.C., and Vaillet, J., *American Society of Hematology* 226-230 (2005) (Exhibit B). Shore and Viallet report that such apoptosis

suppressors (Bcl-2, Bcl-xL, Bcl-w, Mcl-1 and A1) are often strongly elevated in diverse cancers, and have the potential to confer resistance to endogenous cell death stimuli and many cancer treatments. Moreover, multiple pro-survival members are typically up-regulated in a given cancer. Thus, new compounds are being tested which antagonize multiple Bcl-2 family proteins and induce apoptosis. GX15-070, a small organic molecule which reportedly binds to Bcl-w, Bcl-xL and Mcl-1 with a K_D value in the 0.5 μM range, was found to exhibit antitumor activity as a single agent across diverse cancer cell types. (See pages 228-229 of Shore and Vaillet.) Thus, a small organic molecule which binds multiple Bcl-2 family members is expected to be useful for the treatment of diverse cancer cell types.

4. (-)-Gossypol binds to Bcl-2 and Bcl-xL with quite high affinities. (-)-Gossypol has K_i values of 320 nM and 480 nM to Bcl-2 and Bcl-xL proteins, respectively, in a competitive fluorescence polarization-based binding assay. A competitive binding assay showed that (-)-gossypol also binds to Mcl-1 protein, another anti-apoptotic Bcl-2 member, with a K_i of 180 nM. Thus, (-)-gossypol binds with high affinity to three apoptosis-suppressing members of the Bcl-2 family and, like GX15-070, is expected to be useful for the treatment of diverse cancer cell types.

5. (-)-Gossypol has been tested in the clinic for cancer treatment activity as a single agent in a clinical trial. As shown in Exhibit C, when (-)-gossypol (AT-101) was administered to patients with chronic lymphocytic leukemia (CLL),[1] activity was observed in terms of reduced cancer cell load in the peripheral blood (6 of 8 patients),

[1]In this study, CLL patients received a total dose of 30 or 40 mg of (-)-gossypol either once daily or in divided doses either every day or for 21 of 28 days for a maximum of 12 weeks.

lymph nodes (8 of 8 patients) and spleen (6 of 6 patients with an enlarged spleen). In addition, one patient exhibited an improved platelet count and 2 patients exhibited improved disease symptoms. While these patients were not cured, they most certainly have been treated.

6. (-) Gossypol is also being tested in the clinic for activity against prostate cancer. In an ongoing study, as of 24 August 2006, two of twenty-three patients treated have experienced partial responses of the tumor marker prostate specific antigen (PSA), an indicator of clinical activity. Five others have experienced at least some improvement in PSA levels. Ten patients remain on treatment, including the two with partial PSA responses, while the others have discontinued treatment for progressive cancer or other reasons. For those patients who exhibited a partial response, their prostate cancer was certainly treated."

7. In another study, 30 mg/day of (-)-gossypol was administered to a patient for the treatment of follicular lymphoma. As shown in Exhibit D, the patient experienced a 61% reduction in lesion size.[2] For this patient, the follicular lymphoma was certainly treated.

8. Additional evidence which supports the enablement of the present invention may be found in published U.S. Patent Application 20040214902, attached hereto as Exhibit E.

9. On page 5 of the Office Action, it is asserted that the claims are extremely broad insofar as they are directed to the treatment of different cancers with different

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[2]In this study, the patient was treated with rituxamib for one year, then stopped. After about a month, the patient experienced progressive disease. The patient then

etiologies and treatment regimes. However, the breadth of the present claims is justified in view of what is known about strong elevation of the prosurvival Bcl-2 members in diverse cancer cell types, the fact that (-)-gossypol strongly binds to 3 members of this group, and the fact that (-)-gossypol has been found useful for the treatment of multiple types of cancer.

10. On pages 5-6 of the Office Action, it is asserted that no direction or guidance for the administration regimes necessary to treat all of the cancers with the various compounds are given. Further, it is asserted that the working examples are limited to the treatment of adrenal cancer with racemic gossypol and, thus, the Applicants have only provided specific direction or guidance for the treatment of adrenal cancer with racemic gossypol. I respectfully disagree with this assessment. Table 4 of the reissue application provides specific guidance as to dose ranges of (-)-gossypol (20-100 mg/day) that may be administered. These dose ranges encompass the doses of (-)-gossypol which have been found in practice (see above) to be useful for the treatment of various cancers with various etiologies by daily dosing as disclosed in the reissue application. Thus, the present application provides enough direction and guidance for one of ordinary skill in the art to practice the invention without undue experimentation.

11. The Office Action also alleges that the practice of the invention is unpredictable, and that the therapeutic index for gossypol is extremely narrow. As the above clinical studies indicate, the therapeutic index for (-)-gossypol is wide enough to allow for the treatment of a number of different cancers with different etiologies.

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received (-) gossypol. SPD = the sum of the products of the greatest perpendicular diameter of the measured lesion (a measure of tumor volume). LN = lymph nodes.

12. In summary, all of the evidence to date provides the expectation that (-)-gossypol is useful for the treatment of diverse cancers. Accordingly, one of ordinary skill in the art would have every reason to expect that the method of treating cancers as claimed is enabled for its full scope.

13. I have read and understand 37 C.F.R § 10.18(b)

Respectfully submitted,

Signature Date 8/24/06

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CURRICULUM VITAE

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MEDICAL LICENSURE: Maryland (D35450)—Inactive

SPECIALTY BOARD CERTIFICATION: Medical Oncology, 1991
Internal Medicine, 1987

BRIEF CHRONOLOGY OF EMPLOYMENT:

4/04-Date	Chief Medical Officer and Vice President, Development Ascenta Therapeutics, Inc. San Diego, CA
3/03-3/04	Vice President, Development Head of Clinical Development Isis Pharmaceuticals, Inc. Carlsbad, California
6/02-3/03	Vice President, Drug Development Isis Pharmaceuticals, Inc. Carlsbad, California
3/00-6/02	Executive Director, Drug Development Isis Pharmaceuticals, Inc. Carlsbad, California
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8/97-12/97	Associate Medical Director, Drug Development Isis Pharmaceuticals, Inc. Carlsbad, California
1/96-8/97	Senior Investigator Biological Resources Branch, Developmental Therapeutics Program, and Investigational Drug Branch, Cancer Therapy Evaluation Program Division of Cancer Treatment, Diagnosis, and Centers National Cancer Institute National Institutes of Health Frederick, Maryland and Rockville, Maryland

7/90-12/95	Project Officer, Program Director, and Senior Clinical Investigator Biological Resources Branch and Clinical Research Branch Biological Response Modifiers Program Division of Cancer Treatment National Cancer Institute National Institutes of Health Frederick, Maryland
7/88-6/90	Fellow in Hematology/Oncology George Washington University Medical Center Washington, D.C.
7/87-6/88	Instructor in Internal Medicine George Washington University Medical Center Washington, D.C.
7/84-6/87	Resident in Internal Medicine George Washington University Medical Center Washington, D.C.
7/81-6/82	Aide to Senator Pete V. Domenici (R-NM) United States Senate Washington, D.C.

PROFESSIONAL SOCIETIES: American Society of Clinical Oncology
American Association for Cancer Research
Christian Medical/Dental Society

HONORS/AWARDS: Sustained Superior Performance Award
Department of Cancer Treatment, Diagnosis, and Centers
National Cancer Institute
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Department of Cancer Treatment
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COMMITTEES/OTHER ACTIVITIES:

Fall 1995-Spring 1997 — Core Courses in Biochemical Regulatory Engineering Program University of Maryland, Baltimore County:

Regulatory Issues in Biotechnology	Fall 1995
Good Manufacturing Practices for Bio-Processes	Spring 1996
Quality Control and Quality Assurance of Biotechnology Products	Fall 1996
Biotechnology GMP Facility Design, Construction and Validation	Spring 1997

June 11-13, 1997 — "The Mechanics of Preparing INDs and NDAs and FDA Regulations"
Institute for Applied Pharmaceutical Sciences
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1994-1997 — Protocol Reviewer for Physician's Data Query

1991-1997 — Reviewer for Journal of the National Cancer Institute

1990-1997 — Biologics Operating Committee, DCTDC/NCI/NIH

1990-1997 — Decision Network Committee, DCTDC/NCI/NIH (AD HOC)

1991-1992 — Organizing Committee for FDA/NCI Workshop on Preclinical Safety Testing of Monoclonal Antibodies (January, 1992, Bethesda, Maryland)

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BIBLIOGRAPHY

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Abstracts

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Modulating the Bcl-2 Family of Apoptosis Suppressors for Potential Therapeutic Benefit in Cancer

Gordon C. Shore and Jean Viallet

Members of the BCL-2 family of proteins regulate and execute many cell intrinsic apoptosis pathways, including those arising from dysregulated expression of cellular oncogenes. Since pro-survival members of the family are often strongly elevated in diverse cancers, with the potential to confer resistance to both endogenous cell death stimuli and many cancer treatments, there has been intense interest to develop strategies to therapeutically modulate their activity. Although encouraging genetic and pharmacological preclinical proof of concept has been obtained, the challenge for clinical development will be to devise strategies that address the fact that multiple pro-survival members are typically up-regulated in a given cancer and the family operates primarily through protein-protein interactions. Moreover, since several current therapies themselves are known to stimulate the levels of one or more family members, there will be additional challenges (and opportunities) in exploiting this target in the clinic. In this review, we describe the rationale for targeting the BCL-2 family of apoptosis suppressors in cancer and the progress that has been made in modulating the family by small molecule antagonists.

Apoptosis is believed to have evolved in metazoans to regulate tissue homeostasis and to eliminate individual cells that have become superfluous, have become dysfunctional due to infections, or sustained chromosomal alterations that could subvert normal growth control. Apoptosis therefore provides a defense against numerous assaults that could otherwise inflict damage or kill the organism. During oncogenesis the cell must bypass these inherent apoptosis mechanisms if the cancer cell is to survive because apoptosis is otherwise triggered by both the aberrant growth pattern of these cells and the application of many cancer therapies. Cancer cells can evade apoptosis in either of two ways: by inactivation of genes or gene products that promote apoptosis (e.g., the p53 tumor suppressor gene) or by activation of inhibitors of cell death pathways (e.g., the BCL-2 family of apoptosis suppressors). Therapies that target these regulators and re-instate the normal apoptotic mechanisms in cancer cells hold significant promise.[1-3]

Oncogenes as Inducers of Apoptosis Pathways

Unrestricted mitogenic stimuli arising from dysregulated oncoprotein signaling is an early step in conferring a predisposition to malignant transformation, a condition that is normally held in check by interlocking tumor suppressor mechanisms, usually resulting in apoptosis.[4,5] The concept that transforming oncogenes can stimulate apoptosis mechanisms is now well established in many contexts, and includes cell membrane signaling by Ras[6] or transcriptional changes effected by Myc.[7,8] Animal models have been engineered for pancreatic beta cell oncogenesis in which a combination of c-Myc expression and upregulation of a suppressor of apoptosis, BCL-X_L, is both necessary and sufficient to permit c-Myc-induced initiation and progression of cells into angiogenic, invasive tumors.[9] Conditional activation of c-Myc in adult, mature beta cells in this transgenic mouse model in the absence of an apoptosis suppressor, on the other hand, induced uniform beta cell proliferation but was accompanied by massive apoptosis, which rapidly degraded the beta cell mass. Conversely, in a mouse lymphoblastic leukemia model driven by constitutive c-Myc[10] and conditional BCL-2 expression, subsequent elimination of BCL-2 yielded rapid loss of leukemic cells and significantly prolonged survival. After turning off BCL-2, the oncogenic potential of c-Myc was overcome by apoptosis, formally validating inhibitors of BCL-2 as a rational strategy for therapeutic development. Based on these and related findings it has been proposed that a combination of dysregulated cell proliferation and reduction in apoptosis is key for the development of cancer, with the secondary traits of diverse neoplasms resulting as outcomes of this platform.[9,11]

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Acknowledgments: We thank Pierre Beauparlant for reviewing the manuscript and providing input.

BCL-2 Family of Death Regulators

The BCL-2 family is central to both the regulation and execution of most intrinsic apoptotic pathways.[12] The family is comprised of 3 groups, classified according to their content of BCL-2 homology (BH) domains (for a recent detailed review, see [12]). Anti-apoptotic members (e.g., BCL-2, BCL-X_L, BCL-w, MCL-1, and A1) contain four BH domains defined by their similarity among the members of the family; the multi-BH domain pro-apoptotic members BAX and BAK contain BH domains 1-3; and a diverse group of loosely related pro-apoptotic proteins (e.g., BID, BAD, BIM, BIK, PUMA, NOXA, etc.) contain only BH domain 3 (BH3). All anti-apoptotic members as well as BAX and BAK contain a hydrophobic transmembrane (TM) domain located at their extreme C-terminus, whereas among BH3-only members BIK, BIM, and PUMA contain a C-terminal TM. Anti-apoptotic members have the potential to hetero-dimerize with pro-apoptotic members through binding of the exposed BH3 helix on the surface of pro-apoptotic members into a deep groove on the surface of anti-apoptotic members, formed by helices 1 and 2.[13] BAX and BAK, as well as certain BH3 only proteins, undergo a conformational change in response to upstream death signaling pathways, resulting in exposure or availability of the BH3 domain. Heterodimerization with anti-apoptotic BCL-2 members, therefore, typically occurs with activated pro-apoptotic conformers.[14-16] Of note, a number of BH3 only proteins, including PUMA, NOXA and BIK exist as constitutively active conformers and therefore their contribution to death signaling necessarily involves new protein synthesis. In addition, certain BH3-only proteins can interact, at least transiently with BAX or BAK.[14-16] The outcome of death signals that are regulated by the BCL-2 family, therefore, depends upon a complex three-way ratio of the multi-domain anti-apoptotic, multi-domain pro-apoptotic, and BH3-only members (**Figure 1**).

Studies employing double gene deletions of murine Bax and Bak have shown that these two proteins function as essential effector molecules in many death pathways[17] and that the anti-apoptotic BCL-2 and pro-apoptotic BH3-only members operate both upstream and through these effector molecules.[18] The BH3-only members function as proximal sensors of apoptotic stimuli and in their active conformers can bind and inhibit BCL-2 members (e.g., BAD and NOXA) or they can both inhibit BCL-2 members as well as activate BAX and BAK by a "hit-and-run" mechanism (e.g., tBID and BIM). The former act as sensitizers of stimuli that activate BAX and BAK, whereas the latter are both sensitizers and activators.[15]

As depicted in **Figure 1**, the ratio between pro-survival BCL members and pro-death members dictates the outcome of many death-initiating signalling pathways. To achieve this, the BCL-2 family functions at two sites within the cell: mitochondria, where the BCL members regulate the release of factors from the organelle that activate caspases and remodel chromatin; and endoplasmic reticulum (ER), where the BCL members regulate ER Ca^{2+} homeostasis and release.[19] The BCL-regulated ER Ca^{2+} pathway is linked to the mitochondria, causing morphological and structural transitions that allow mitochondria to respond to pro-apoptotic stimuli.[20] Of note, pro-survival BCL members are differentially enriched at mitochondria and ER.[21] **Figure 2** (see Color Figures, page 552) illustrates the pathway at mitochondria, in which an oncogenic stimulus results in activation of one or more BH3-only members, which can target and antagonize pro-survival members. Productive antagonism of pro-survival members either alone or coupled with stimuli to directly activate BAX and BAK, results in the oligomerization of BAX or BAK. This allows the formation of a predicted conduit for release of pro-apoptotic factors such as cytochrome c, a co-factor that results in activation of the apoptosome, which in turn activates effector caspases –3 and –7.[12]

Not All BH3 Domains Are Created Equal

Individual BH3-only BCL-2 members appear to have evolved both to link specific upstream signals to downstream activation of the mitochondrial apoptosis pathway and to selectively target preferred BCL-2 binding partners. For example a recent study of the affinity of 8 BH3 peptides for the soluble forms of 5 pro-survival BCL-2 proteins, employing a Biacore Biosensor, revealed a 10,000-fold range in binding affinity.[22] BIM and PUMA, for example, exhibited similar affinities for all pro-survival members whereas NOXA bound only to MCL-1 and A1. BH3-only BIK, which can be induced by oncogenic stress, preferentially targets the ER where it binds pro-survival members and initiates Ca^{2+}-mediated remodelling of mitochondrial cristae, mobilizing mitochondrial stores of cytochrome c as a prerequisite for its release to the cytosol.[20] Interestingly, BIK and NOXA cooperate to release cytochrome c.

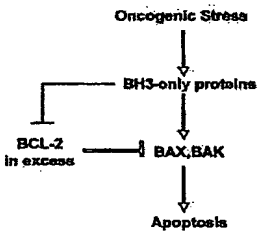

Figure 1. Model for regulation of oncogene-driven apoptosis by BCL-2 family proteins. Oncogenic stress pathways lead to activation of several BH3-only proteins. They all antagonize pro-survival BCL-2 members but in addition certain of these BH3-only members also activate BAX and BAK. When in excess, pro-survival members prevent apoptosis by antagonizing the activated conformers of BAX and BAK. Therapeutics that shift the balance in favor of excess antagonists of pro-survival members should permit oncogenic stress pathways to productively engage the apoptosis mechanism. Details are given in the text.

Likewise, BAD and NOXA, which have non-overlapping binding preferences for pro-survival members, cooperate to induce cell killing.[22] From a therapeutic perspective, it might be expected that mimetics of the BH3 domains of BIM and PUMA exhibit pan-BCL inhibition, whereas more selective antagonists might be generated from other BH3 mimetics, such as those derived from BAD or NOXA.

Pro-survival BCL-2 Proteins: Therapeutic Targets

Since pro-apoptotic BCL family proteins dock into the BH1-BH2 groove of pro-survival members via their BH3 domain, it has been proposed that BH3 mimetics that antagonize the pro-survival members could be used to alter the ratio between pro-survival and pro-apoptotic members, allowing apoptosis to proceed in cancer cells. Strong support for such a strategy has come from the findings that BH3 peptides themselves can inhibit BCL members, induce apoptosis in cancer cell lines, and in one case where the pharmacological properties of the peptide were improved, induce apoptosis in a mouse xenograft tumor model.[23] In this latter case, a chemical strategy termed hydrocarbon stapling was used to stabilize the alpha-helical BH3 peptide derived from the BH3-only protein BID. BID is strongly pro-apoptotic and belongs to the class that activates BAX and BAK as well as antagonizes pro-survival BCL members.[23] The stapled peptide proved to be cell permeant and protease-resistant, and interacted with pro-survival members with increased affinity. It was also effective in inhibiting the growth of human leukemia xenografts in SCID mice. Thus, a BH3 peptide, and therefore small molecules that mimic this domain, has the potential to therapeutically modulate BCL-2 family proteins.

Therapeutic Small Molecule Discovery Strategies

The challenge of this strategy is to discover corresponding small molecule BCL antagonists with drug-like properties. Moreover, because of the complexity of BCL proteins in cancer cell biology, including upregulation of multiple family members in a single cell and the contribution of different family members to mitochondria- and ER-regulated pathways, a small molecule antagonist of multiple pro-survival members is likely required, i.e., a pan-BCL-2 inhibitor, at least for initial proof of concept studies in the clinic. Although a number of such antagonists are currently at various stages of development, we focus below on two examples representing distinct discovery strategies: rational design and functional screening.

ABT-737

One approach is based on rational design and high throughput SAR by NMR.[23] Utilizing the high resolution structure of the BH3 docking groove on the surface of the pro-survival BCL member, BCL-X_L,[13] inhibitors of BCL-X_L, BCL-2, and BCL-w were generated by covalently bridging chemical entities that bind to separate regions of the groove. One, ABT-737, exhibits an affinity for these targets in vitro 2- to 3-orders of magnitude more potent than the multiple small molecule antagonists that have previously been reported in the literature ([24] and references cited therein). Of note, however, it exhibits significantly reduced affinity for MCL-1, a BCL member whose structure is intermediate between the "closed" conformation of unliganded BCL-2/BCL-X_L and their more "open" BH3-complexed conformers.[25] A number of amino acids in the binding groove also distinguish MCL-1 from other members. Nevertheless, ABT-737 demonstrated potent single-agent killing of select cell lines from small cell lung carcinoma and lymphoma, and against peripheral blood mononuclear cells (PBMNCs) derived from 7 of 13 patients with chronic lymphocytic leukemia (CLL). From a mechanistic perspective, ABT-737 appears to fall into the class of BH3 "sensitizers," since it fails to directly activate BAX or BAK and release cytochrome c from mitochondria in vitro.[24] Despite the relatively large size of the compound, ABT-737 achieved potent anti-tumor activity in mouse H146 and H1963 small cell lung carcinoma (SCLC) mouse xenograft models when administered i.p. at 75-100 mg/kg daily for 3 weeks.

GX15-070

An alternative discovery approach is based on functional outcomes and seeks small molecules that inhibit BCL protein-protein interactions. Since BCL members have the potential to undergo conformational changes,[14,16,27] these assays accommodate the possibility of dynamic changes in protein structure contributing to these interactions. Thus, a high throughput protein-protein interaction discovery screen was used to interrogate natural compound libraries, which identified a chemotype that falls within the polypyrrole class of molecules[28] as a starting point for optimization. Further development resulted in the compound GX15-070, a non-prodigiosin, which is currently in clinical development.

[^{3}H]-labelled GX15-070 was found to bind to BCL-w, BCL-X_L, and MCL-1 with K_D values in the 0.5 μM range. In contrast to ABT-737, therefore, GX15-070 appears to bind pro-survival members as purified entities in vitro with apparent reduced affinity. However it also targets MCL-1. After exposure of sk-MEL5 melanoma cells to GX15-070 for 5 hours and detergent extraction, interaction between MCL-1 and BAK was inhibited relative to vehicle controls, as judged by co-immunoprecipitation, with an apparent IC_{50} of about 1.5 μM.

To formally prove that GX15-070 can antagonize pro-survival BCL members, resulting in activation of BAX or BAK, the BCL pathway was engineered into yeast cells. *S cerivisiae* does not express BCL-related proteins and is not sensitive to GX15-070-mediated cytotoxicity. In contrast, overexpression of BAK in these yeast is cytotoxic, but can be countered by pro-survival members BCL-w, MCL-1, or BCL-X_L. However, treatment of the yeast cells with GX15-070 was toxic, an effect dependent on the presence of BAK suggesting that GX15-070 can antagonize the pro-survival

BCL proteins, overcoming their ability to inhibit BAK. Consistent with this mechanism, when transformed baby mouse kidney epithelial cells expressing adenovirus E1A and dominant-negative p53 and derived from either wt mice or mice doubly deleted of *Bax,Bak*, the double knock out cells resisted the activation of caspases by GX15-070. As expected, treatment of cancer cell lines with GX15-070 resulted in oligomerization of mitochondrial BAK, release of cytochrome c, and activation of caspases. Collectively, the results suggest the mechanism illustrated in **Figure 2** (lower panel; see Color Figures, page 552) for the activation of caspases by GX15-070.

Further testing showed that GX15-070 exhibits single agent cytotoxicity against a broad range of cell lines and ex vivo against PBMNCs from patients ($n > 30$) with CLL. Delivery of formulated drug by intravenous bolus injection into the tail veins of Balb/c or CB17 SCID/SCID mice daily for 5 consecutive days was well tolerated, and in animals pre-implanted subcutaneously with cell lines derived from cervical (C33A), colon (SW480), prostate (PC3), or mammary (4T1) carcinomas and allowed to form palpable tumors, administration of GX15-070 on this schedule resulted in inhibition of tumor growth relative to vehicle alone. For example, at 2 mg drug/kg body weight given daily for 5 days, inhibition of growth of these tumors ranged from 60%-85% 14 days after initiating the administration of drug, with no weight loss observed in the animal cohorts. Thus, as predicted from the mechanism of action of BCL proteins and their ability to antagonize oncogenic apoptotic pathways, GX15-070 exhibits antitumor activity as a single agent across diverse cancer cell types.

Phase I evaluation of GX15-070, administered by intravenous infusion on an every 3 week schedule in patients with refractory CLL and weekly in patients with refractory solid tumors, is in progress.

Pharmacodynamic Markers for BCL-2 Mechanism-Based Cancer Treatments

Evidence of mechanistic and biological activities of BCL mechanism-based therapies can be obtained by measuring these activities directly in cancer cells isolated from the patient as well as by measuring surrogate markers released into the circulation. As indicated, PBMNCs isolated from patients with CLL and incubated with GX15-070 or ABT-737 ex vivo underwent apoptosis. In the case of GX15-070, evidence of disruption of interactions between MCL-1 and BAK was observed following cell extraction in detergent and co-immunoprecipitation. Similar protein-protein interaction assays can be performed on circulating leukemia cells isolated from patients at timed intervals after receiving the drug by intravenous administration. Additionally, end products of apoptosis such as chromatin fragments can be detected following their release into the circulation,[29] thereby serving as a surrogate of tumor cell death. Collectively the results of such biological measurements can be exploited to optimize dose and schedule of drug administration.

Rational Combination Treatments

Since BCL-2 proteins confer resistance to most cell death stimuli that operate through the mitochondria apoptosis pathway, it is predicted that a number of current cytotoxic cancer treatments might benefit from combination therapy with BCL-2 antagonists. For example, among others, ABT-737 has been reported to enhance the cytotoxicity of paclitaxel in A549 NSCLC cells.[26] Additionally, however, certain current therapies themselves can directly influence the expression of BCL-2 family proteins. The proteasome inhibitor Velcade® (Bortezomib, PS-341; Millennium Pharmaceuticals) is currently approved for the treatment of multiple myeloma and is in development for other indications.[30] By blocking ubiquitin-mediated protein degradation, Bortezomib is predicted to interfere with, among others, survival mechanisms associated with nuclear factor (NF)-κB pathways. It has also been shown to cause elevation of BH3-only NOXA,[31,32] a preferred binding partner for MCL-1.[22] However, at steady state the turnover of MCL-1 is rapid, and this protein also is subject to ubiquitin-mediated degradation via the proteasome.[33] Indeed, proteasome inhibitors can lead to a rapid increase in MCL-1 protein levels in various cell lines within several hours of treatment. If the rise in anti-apoptotic MCL-1 is not offset by pro-apoptotic NOXA (or other BH3 ligand), then a combination of Bortezomib and an effective small molecule antagonist of MCL-1 may prove beneficial.

Conclusions

The complex interplay between multiple BH3-only proteins and their pro-survival binding partners raises both challenges and opportunities in devising small molecule BH3 therapeutic mimetics for the treatment of cancer. Since more than one pro-survival member is typically overexpressed in a given cancer, early exploration of preclinical and clinical proof of concept is focusing on small molecule antagonists that target multiple pro-survival members. As our understanding of the role of individual pro-survival members in cancer signalling improves, it may prove desirable in certain contexts to design more selective antagonists. The fact that different BH3-only proteins have evolved to accomplish such selectivity suggests that this may indeed be feasible. Coupled with this, however, will be the need to better understand the differential contributions that individual pro-survival members make to cancer-related apoptosis pathways, and to devise the pharmacodynamic and biomarker tools necessary to exploit these opportunities clinically. The clinical development of first generation BCL-2 family antagonists may teach us valuable information about the most effective way to modulate this important family of apoptosis suppressors.

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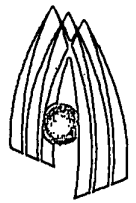
AT-101 in 1st-line, High Risk CLL (Kipps/UCSD)

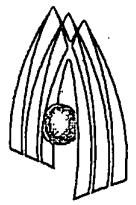
James et. al., abstract #6605, ASCO 2006



- Previously untreated CLL patients
  - High risk features
- Single Agent AT-101 at 30mg – 40 mg daily for 12 weeks
- Evidence of activity in all disease compartments
  - Peripheral blood (6 of 8 patients)
  - Lymph nodes (8 of 8)
  - Spleen (6 of 6 with enlarged spleen)
  - Improved platelet count (1 patient)
  - Improved disease symptoms (2 patients)
- Early evidence of pharmacodynamics

Lymphoma - Partial Response Summary

Patient 02003 on Protocol AT-101-CS-005



- **May, 2004**
  - Diagnosed with Follicular Lymphoma
- **Nov, 2004**
  - Treated with Rituximab for 12 months
- **Jan-6, 2006**
  - Progressive Disease
- **Jan-31, 2006**
  - Enrolled on Protocol
  - 30mg daily until Mar-27
  - Intermittent interruptions due to toxicity (skin rash/nausea/vomiting/diarrhea)
- **Mar-28, 2006**
  - Decreased to 30mg for 3 of each 4 weeks
- **May-2, 2006**
  - Self withdrawal from protocol
  - Due to grade 1 nausea and prior experience with GI toxicity (n/v/d)

Response Measurement Summary

Clinical Assessment	Baseline	8 Weeks	% Change
SPD of LN (per CT)	10.92 cm^2	4.25 cm^2	-61.1%
LDH	345	340	-1%
Bone Marrow	Normal	N/D	N/A
Spleen	Normal	Normal	N/A
Liver	Normal	Normal	N/A